International Journal of Tropical Medicine, 1(2) : 53-57, 2006

A Cross-sectional Study of *Blastocystis hominis* in Primary Schoolchildren, Northwest Iran

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Abstract: *Blastocystis hominis* is a protozoan parasite whose importance as a cause of gastrointestinal pathology is controversial. The aim of this study was to evaluate the frequency of *Blastocystis hominis* (*B. hominis*) and the presence of symptoms among primary schoolchildren. This cross-sectional study was conducted in 1070 schoolchildren between 7-13 years old in northwest Iran, 2003. A questionnaire was completed for each child. Stool specimens were collected by stratified random sampling and were examined using direct wet mount for presence of *B. hominis* and formalin-ether concentration method for helminthes eggs and protozoan cysts. The most common parasite was *B. hominis* (<5 cells per field) (28.2%) followed by *Giardia lamblia* (*G. lamblia*) 14.2%. A total of 109/1070 (10.2%) showed ≥5 *B. hominis* per field. The most common symptoms in children who showed only *B. hominis* were abdominal pain (49.4%), anorexia (35.8%) and nausea (33%). On the basis of our findings we recommend that *B. hominis* be considered as a potential pathogen in symptomatic people, especially children when the number of organisms in a stool specimen exceeds five organisms per field. Awareness on the part of clinicians and laboratory technicians is essential for proper diagnosis and treatment of cases.

Key words: *Blastocystis hominis*, schoolchildren, Iran, protozoam parasite

INTRODUCTION

B. hominis, a protozoon whose pathogenicity has already been much debated, is sometimes found in the gastrointestinal tract[1-3]. Its presence has been reported in a wide variety of intestinal disorders resembling Irritable Bowel Syndrome (IBS) such as bloating, flatulence, mild to moderate diarrhea, abdominal pain and nausea[4-9]. Association of this parasite with epidemics of diarrhea in tropical and subtropical countries has been reported[10-13]. The severity of clinical symptoms increased whenever the number of parasites was more than five trophozoites per field at a magnification×400[14]. The purpose of this study was to determine the prevalence and clinical significance of *B. hominis* among schoolchildren in northwest Iran.

MATERIALS AND METHODS

This cross-sectional study was conducted in 1070 primary schoolchildren between 7-13 years old in 20 schools in northwest Iran, 2003. A questionnaire was completed for each student. The questions covered age, sex, personal hygiene, education and occupation of parents. All individuals were asked to provide one stool sample in disposable stool boxes for analysis. Samples were sent to the Department of Parasitology and Microbiology, School of Medicine, Ardabil University of Medical Sciences. All portions of stool specimens were initially examined directly for the presence of *B. hominis* by preparing a standard unstained wet mount in physiologic saline (0.85%)[15] and a stained mount by iodine and then reexamined after concentrating them in formalin ether for the detection of helminthes eggs and protozoan cysts. Samples were considered positive for *B. hominis*; if any vacuolar, granular, or amoeboid forms of *B. hominis* were detected. *B. hominis* was considered pathogen if≥5 organisms per×400 field were detected. The positive rates were expressed as percentage and the data obtained were analysed with the Statistical Package for Social Science (SPSS) by using Chi-square test for qualitative variables. A p-value less than 0.05 was considered significant.

RESULTS

B. hominis could be detected easily in the iodine wet mount without concentration. Two forms of trophozoites, granular and vacuolated, appeared in the same stool

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Table 1: Prevalence rate of intestinal parasites in primary schoolchildren in Northwest Iran, 2003

Parasites	No. of parasites (%)
B. hominis (<5 cells per field)	320 (28.2)
B. hominis (≥5 cells per field)	
-With or without other parasites	109 (10.2)
-alone	85 (7.9)
G. lamblia	152 (14.2)
Commonsal protozoa cysts	61 (5.7)
Helminthes eggs	16 (1.5)

Table 2: *B. hominis* in schoolchildren grouped by grade and region, Northwest Iran, 2003

		B. hominis positive	
Grade (class)	Region	No.	(%)
First	1	5	5.7
	2	14	12.7
Second	1	7	7.1
	2	13	11.2
Third	1	11	11.6
	2	17	3.1
Forth	1	10	11
	2	12	10
Fifth	1	10	9.7
	2	8	7.9

Table 3: Distribution of symptoms in schoolchildren infected with only *B. hominis*, Northwest Iran, 2003

Symptoms	No.of children (%)
Abdominal pain	42 (49.4)
Anorexia	30 (35.3)
Fever	29 (34.1)
Nausea and Vomiting	28 (32.9)
Weakness	19 (22.3)
Diarrhoea	11 (12.9)
Asymptomatic (Carrier)	25 (29.4)

specimens and in some specimens small and large trophozoites with a large central body and granular cytoplasm were seen. Of the 1070 stool examined, 10.2% (109/1070) were found to be positive for *B. hominis* (≥5cells per field). Of course 28.2% (320/1070) showed less than 5 *B. hominis* per field (Table 1).

The positive rates of *B. hominis* in boys and girls were 10.4% (55/472) and 9.9% (54/489), respectively. Statistics found no significant difference in positive rate between boys and girls ($p>0.05$).

Prevalence rate of infection in students grouped by grade has been shown in Table 2. There was a significant difference between students first grade in regions 1 and 2 ($p<0.05$).

Infection rate in children who their mothers were employee was lower than those were housekeeper ($p<0.05$). Table 3 shows the gastrointestinal symptoms in schoolchildren infected with only *B. hominis*.

Abdominal pain was the most common symptom, followed by anorexia. Almost 30% of children showed no symptoms (carrier). Symptoms in individuals infected with *G. lamblia* were quite similar to those infected with *B. hominis*.

DISCUSSION

B. hominis has been traditionally regarded as a non-pathogenic parasite of humans. The pathogenic potential of *B. hominis* has been reported in the literature since 1899.[1] and studies reporting its association with human disease have been increasing[3,16-21]. Its pathogenicity seems to depend on the number of parasites. Reports of symptomatic individuals with fewer than five parasites per ×400 field have also been documented and more sever symptoms have been noticed with an increase in number of parasites[22,23]. Vannatta *et al.*[24] employing this criterion, reported that *B. hominis* is a causative agent of recurrent diarrhea. Because oil immersion is used infrequently in routine examination of direct smears, we chose to investigate individuals with five or more *B. hominis* cells at 40×magnification. Our study suggested that the presence of five or more *B. hominis* cells per 40×field is associated with clinical symptoms in most individuals.

In this study, the prevalence rate of *B. hominis* (≥5 cells per field) in schoolchildren was 10.2% (109/1070), in 7.9% of which *B. hominis* was detected in the absence of other parasites.

Other studies carried out in Iran have reported different rates of this parasite (1.3-28.4%)[25]. The prevalence rate of *B. hominis* in China, United Kingdom, Spain, Thailand and Venezuela was 1-5.9, 6.9, 16.5, 0.8 and 16%, respectively[4,26-29].

Results of this study shows that the prevalence of *B. hominis* in schoolchildren grades 1, 2 and 3 in region 2 was higher than those in region 1 ($p<0.05$). Of course in grade 4 and 5 was the same. This maybe due to lower level of education in grades 1, 2 and 3 than grades 4 and 5 and socio-economic, cultural and hygiene habits differences, in which region 2 is lower than those in region 1.

Prevalence of *B. hominis* in children with employee mothers was less than those with housekeeper mothers ($p<0.05$). This is probably because of the higher level of education in employee mothers than housekeepers ones. Employee mothers play an important role in hygiene education to their children.

In this study, we observed *B. hominis* in 109 of 1070 (10.2%) specimens examined. The 24 specimens that contained other pathogens *G. lamblia* cysts and helminthes eggs) were excluded. The remaining specimens (85/1070) were divided according to the information in the questionnaire into asymptomatic (29.4%) and symptomatic (7.9%) groups.

The most common symptom was abdominal pain (49.4%). Sheehan *et al.*[12,30] also have reported that abdominal pain was the most frequent symptom (88%).

Garcia *et al*.[3] observed that diarrhoea and then abdominal pain were main symptoms. However, in our schoolchildren the second most common complaint was anorexia (35.3%), followed by fever, nausea, weakness and diarrhoea (12.9%).

In this study 29.4% of individuals infected with only *B. hominis* had no symptoms, these carriers are very important in epidemiology and transmission of parasite to other people. Because these individuals do not show any symptoms, do not refer to physicians, pass off continuously the parasite and contaminate environment and other people. So identification and treatment of these people is necessary.

Although the reasons why the organism had been found in both symptomatic and asymptomatic individuals have been largely unknown[31-37], one possibility was that it was due to infection time, infection dose, poly-infection with bacteria and the ability of host immunity that might decide whether the symptom turned up or not, because only over 24 h could the cysts of *B. hominis* develop into a large number of vacuolar forms[38,39].

The distribution of symptoms was quite similar to that seen in children with *G. lamblia*. This subject is very important, because usually laboratories do not report *B. hominis* and physicians also with these symptoms become suspicious of *G. lamblia* that might such as this study, is not detected in specimens. So physicians maybe amazed.

In conclusion, our study supports that *B. hominis* should be considered as a causative agent of human disease in mind of parasitologists and physicians when dealing with patients with diarrhoea especially when the parasite is present in large numbers in fecal specimens in the absence of other known pathogens. *B. hominis* has long been described as a non-pathogenic protozoa parasite[40-42]. Many labs do not know that it is now considered harmful to human or do not know how to test for it. Furthermore, because of absence of specific symptoms, the disease was easily confused with other intestinal disease and was easily misdiagnosed. The authors suggested that stool examination should be carried out on patients with diarrhoea in order to decide whether or not the patients were infected by *B. hominis* and the stool samples should be collected more than once from patients showing clinical signs and symptoms.

ACKNOWLEDGEMENT

I am grateful to all students of Family Health course at which the specimens were collected for their kind help and cooperation. I also thank students of Laboratory Sciences course for technical help.

This work was supported by research grant no.83/221 from Ardabil University of Medical Sciences.

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